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CONSENSUS REPORT

Tissue Handling in Suspected Creutzfeldt-Jakob Disease (CJD) and Other Human Spongiform Encephalopathies (Prion Diseases)

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Despite many sensational and intimidating reports in the mass media, transmissible spongiform encephalopathies (prion disease) are not contagious in the usual sense. Successful transmission requires both specific material (an affected individual's tissue, from or adjacent to CNS) and specific modes (mainly penetrating contact with the recipient). Nevertheless, specific safety precautions are mandatory to avoid accidental transmission and to decontaminate any infectivity. Autopsy is essential for definite diagnosis of these disorders. Recommendations are given here for performance of the autopsy, for neuropathology service and appropriate decontamination; they are based on the current literature and on precautions taken in most laboratories with experience in handling tissue from transmissible spongiform encephalopathies. In particular, special care must be taken to avoid penetrating wounds, possible contamination should be kept to a minimum, and potential infectious material must be adequately decontaminated by specific means.

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"Childbed fever is a transmissible, but not a contagious disease"
(Ignaz Semmelweis: The difference of opinion between the English doctors and myself about childbed fever; 1860, in Hungarian)

What Semmelweis wrote in the 19th century about childbed fever is true today also for spongiform encephalopathies. Despite many sensational and intimidating reports in the mass media, transmissible spongiform encephalopathies (prion diseases) are not contagious in the usual sense. Successful transmission requires both specific material (an affected individual's tissue, from or adjacent to CNS) and specific modes (mainly penetrating contact with the recipient). Thus they are not easily acquired diseases. Nevertheless, specific safety precautions are mandatory to avoid accidental transmission and, equally important and difficult, to decontaminate any infectivity. The recommendations given in this report are based on current literature on the topic and on precautions taken in most laboratories experienced in handling tissue from transmissible spongiform encephalopathies. However, safety guidelines vary between laboratories and countries and are continuously revised and updated as new scientific evidence emerges (1-3).

While transmissible spongiform encephalopathies have also been observed in health care workers (5,6,14,15,17,20), there is no established evidence that this professional group bears a higher risk than other members of the population. Specifically, there is no proven case in which a transmissible spongiform encephalopathy developed in relation to contact with patients. In contrast, it seems much more likely to contract other infections, e.g. tuberculosis, HIV or hepatitis, in the professional setting. Moreover, it is notable that numerous

research laboratory personnel working with human and animal transmissible spongiform encephalopathies have yet to produce a single case of accidentally transmitted disease (7).

Diagnosis

What criteria mandate a suspicion of Creutzfeldt-Jakob disease (CJD)? While it is out of the scope of this report to elaborate on clinical diagnosis, the following diagnostic criteria have been utilised by the EC Surveillance Group of Creutzfeldt-Jakob Disease in Europe (Project Leader: R.G.Will, Edinburgh).
Definite CJD is diagnosed by neuropathology (11) and/or, in some laboratories, by additional methodology [prion protein (PrP) Western blot and/or preparation of scrapie-assciated fibrils (SAF)].
In clinical practice, CJD is diagnosed as probable or possible according to the following scheme.

1. **Sporadic CJD**

1.1. **Probable CJD**

Progressive dementia, and
Typical EEG
At least two out of four clinical features listed:
a) Myoclonus
b) Visual or cerebellar disturbance
c) Pyramidal / extrapyramidal dysfunction
d) Akinetic mutism

1.2. **Possible CJD**

Same as 1.1. but without EEG or with atypical EEG and duration of less than two years

2. **Accidentally transmitted CJD**

Progressive cerebellar syndrome in a pituitary hormone recipient
Sporadic CJD with a recognised exposure risk (e.g. dura mater transplant)

3. **Familial CJD**

Definite or probable CJD plus definite or probable CJD in a 1st degree relative
Neuropsychiatric disorder plus disease-specific PRNP mutation

Infectivity of Materials

Which material from human patients is potentially infectious? Known human-to-human transmission in spongiform encephalopathies has been restricted to kuru of the formerly cannibalistic Fore population of Papua-New Guinea, and to iatrogenic inoculation with infected material (intracerebral EEG electrode, dura mater grafts, corneal transplants, pituitary hormones derived from human material). As a result, most data on potential infectivity in these diseases do not derive from transmission to humans, but rather from experimental CJD, scrapie or bovine spongiform encephalopathy (BSE) transmission to primates, rodents or other species.
Tissues and body fluids were classified into 4 classes of infectivity according to specific measures taken by the EU (13) to prevent spread of animal-derived disease [this classification, however, differs from that of the Advisory Committee on Dangerous Pathogens (3)]:
- high infectivity: CNS tissues (brain, spinal cord) and adjacent tissues (eyes);
- medium infectivity: lymphoid tissues (spleen, lymph nodes, tonsils), internal organs with lymphatic component (e.g. ileum, proximal colon), placenta, pituitary gland, dura mater and CSF;
- low infectivity: peripheral nerves, bone marrow, liver, lung, pancreas, thymus;
- no detectable infectivity: muscle, kidney, fat, bone, blood, milk, bile.

The Autopsy

What specific precautions should be made for an autopsy performed on a patient with suspected transmissible spongiform encephalopathy? Since procedures given below can be followed without imposing disproportionate hardship, there is no reason to refuse an autopsy on a patient with suspected transmissible spongiform encephalopathy. Moreover, obtaining the autopsy for establishment of the definite diagnosis is of utmost importance not only for research into these enigmatic diseases, but also for epidemiological and public health reasons. The risk of infection for the personnel can be considered lower than that in autopsies on patients with viral hepatitis or HIV infection, so that the general precautions taken for such autopsies are more than adequate on patients with CJD. Decontamination of the autopsy material, however, requires specific measures. The following protocol is kept as simple as possible; other and more detailed instructions may be found elsewhere (3,4,7,12,16).
The most important objective of the autopsy is to enable a definite diagnosis by neuropathology. It is thus sufficient, in most cases, to restrict the autopsy to removal of the brain. This can be safely done in any autopsy room, does not require specific safety or containment facilities, and does not expose the involved personnel, including morticians etc., to an increased risk, provided that the following procedures are correctly performed.
1. The autopsy personnel should avoid accidental penetrating wounds by wearing protection gear as in other infectious autopsies (hepatitis, HIV infection, etc.), including safety gloves (e.g. teflon-made from Spectra", or metallic gloves) beneath rubber gloves, a disposable apron, and eye and mouth protection.
2. Contamination of the autopsy table should be avoided by a non-permeable disposable plastic sheet or similar material.

3. Removal of the brain: The head is positioned in the usual way, with a thick layer of cellulose sheets underneath. The skull should be opened with a mechanical (non-electrical) hand saw which is easier to decontaminate than an electrical saw. Before removal of the brain from the skull, it is strongly recommended to take some parts of the brain (recommended: apricot-sized pieces of cerebrum, preferentially from one pole, and of cerebellum) for freezing, to enable eventual further diagnostic procedures and research if appropriate. This tissue must be put into two layers of small plastic bags and put into, and frozen within, a tightly closing plastic container which is clearly marked. The brain is then removed from the skull in the usual way and put in a tightly closing plastic container with buffered 4% formaldehyde solution for neuropathological processing. Both the fixed brain and the fixative solution are still infectious and should be appropriately labelled.
4. If wished, internal organs may be inspected and sampled in situ without removal from the body cavities.
5. After the autopsy is completed, the plastic and cellulose sheets are folded together and put with other disposable material into a container for infectious hospital waste to be incinerated.
6. All instruments (saw, knives, etc.) and eventually contaminated surfaces must be decontaminated by procedures given below.
7. Accidents involving parenteral exposure to material or contaminated wastes from transmissible spongiform encephalopathies should be recorded (3).

Neuropathology Service

1. The personnel should wear safety gloves; special care should be taken to avoid accidental penetrating wounds.
2. The formaldehyde-fixed brain is processed on a table covered by a disposable plastic sheet and cut on layers of cellulose sheets (to be discarded by burning with infectious hospital waste).
3. To deactivate CJD infectivity (10), it is recommended to soak the tissue blocks for histology (not more than 5mm in thickness) in concentrated (95-100%) formic acid for one hour, followed by fresh 4% formaldehyde solution for at least 48 hours. However, this makes the tissue block brittle and more difficult to cut. Without this step, paraffin blocks may still be infectious (9).
4. All instruments, gloves etc. which came into contact with potentially infectious material must be decontaminated. Instruments that were contaminated by formaldehyde-fixed, non-formic acid-treated tissue are not decontaminated by autoclaving (19) but should be immersed in 2N NaOH for one hour.
5. Tissue remnants, cutting debris and contaminated formaldehyde solution should be discarded within a plastic container as infectious hospital waste by burning.
6. Accidents involving parenteral exposure to material or contaminated wastes from transmissible spongiform encephalopathies should be recorded (3).

Decontamination

Which decontamination measures must be followed for surfaces, instruments, gloves and other devices that got in contact with tissue material and body fluids from patients with transmissible spongiform encephalopathies ? Since conventional methods of sterilisation and desinfection do not decontaminate the CJD infectious agent (8), specific measures must be used. Several laboratories have developed and use differing but nevertheless effective procedures.

1. Steam autoclaving (glassware, instruments, safety gloves, etc.) 134°C recommended for 1 hour (3,12).

   Comment: Porous load is considered more effective than gravity displacement autoclaves (3). The required time periods have been debated. For porous load autoclaving, only 18 min. at 30lbs psi, or six separate cycles for 3 min. each at 30lbs psi have been recommended (3). However, some laboratories recommend two cycles of autoclaving for 1 hour each, or autoclaving for at least 1 hour during, or subsequent to, soaking in NaOH (see below).
2. Chemical decontamination of nonautoclavable materials and surfaces:

   a) 2N NaOH (80g per litre) for one hour is recommended; alternatively, 1N NaOH may be used for two hours.

   Comment: some laboratories consider mere wiping off as sufficient for surfaces, but others require more extensive washes. Do not use NaOH for aluminium material.

   b) Alternatively 5% NaOCl (at least 20.000ppm free chloride, fresh solution) for 2 hours.

   Comment: very irritating and corrosive for steel.

   c) Some laboratories use boiling of instruments or material in 3% SDS for at least 3min. as another option, either alone or in combination with autoclaving at 121° for one hour (18).

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